Kidney International, Vol. 51 (1997), pp. 216–221

Effect of chronic metabolic acidosis on the growth hormone/IGF-1 endocrine axis: New cause of growth hormone insensitivity in humans

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Effect of chronic metabolic acidosis on the growth hormone/IGF-1 endocrine axis. The effects of metabolic acidosis on growth hormone and IGF-1 are poorly understood. We investigated the effects of chronic metabolic acidosis (induced by administration of NH_4Cl, 4.2 mmol/kg body wt/day) on the growth hormone/IGF-1 endocrine axis in 6 normal male volunteers during metabolic balance conditions. NH_4Cl administration resulted in hyperchloremic metabolic acidosis with plasma bicarbonate decreasing from 25 ± 0.4 to 15.5 ± 0.9 mmol/liter ($P < 0.001$). Metabolic acidosis significantly decreased serum IGF-1 concentration from 45 ± 6 to 33 ± 6 nmol/liter ($P = 0.002$), while serum IGF binding protein 3 concentration was not affected significantly. The growth hormone response to growth hormone releasing factor administration (1 μg per kg body wt, intravenous bolus) was enhanced significantly during acidosis. The IGF-1 response to growth hormone administration (0.1 U kg body wt subcutaneously, every 12 hr for 48 hr) was blunted significantly during acidosis. Apparent endogenous serum half-life and metabolic clearance rates of growth hormone were not altered significantly by acidosis. Metabolic acidosis in humans results in a significant decrease in serum IGF-1 concentration without a demonstrable effect on IGF binding protein 3, and is related to a resistance to the hepatocellular action of growth hormone. The primary defect in the growth hormone/IGF-1 axis occurs via an impaired IGF-1 response to circulating growth hormone with consequent diminution of normal negative feedback inhibition of IGF-1 on growth hormone, as evidenced by the exaggerated growth hormone response to growth hormone releasing factor administration.

Chronic metabolic acidosis results in sustained negative nitrogen balance in humans, which is independent of nutritional deficiencies [1]. The mechanism(s) of acidosis-induced nitrogen wasting is(are) poorly understood in humans. Improved understanding of these mechanisms is important since nitrogen wasting, when prolonged, results in a progressive reduction in lean body mass in a variety of disease states associated with metabolic acidosis (such as end-stage renal disease, cancer and AIDS). This may affect the prognosis of patients with metabolic acidosis adversely since the magnitude of depletion of lean body mass is an effective predictor of the interval to death in such conditions [2, 3].

Growth retardation associated with renal-tubular acidosis in children is reversible upon alkali treatment [4]. In rats, metabolic acidosis has been shown to decrease growth hormone (GH) secretion and insulin-like growth factor (IGF)-1 levels with no significant effects (vs. pair-fed rats) on hepatic IGF-1 and GH receptor mRNA expression [5, 6]. Preliminary data from our previous report suggested that the IGF-1 concentration was reduced during sustained mineral acidosis in normal human subjects [1]. The GH/IGF-1 endocrine axis provides for protein anabolism and enhanced lean body mass, not only during linear growth, but over the entire life span [7, 8]. Therefore, suppression of GH/IGF-1 could mediate the effects of metabolic acidosis on nitrogen balance and, ultimately, lean body mass.

Potassium depletion in rats resulted in reduced serum IGF-1 concentrations and a blunted GH response to growth hormone releasing factor (GRF), suggesting that a hypothalamic/pituitary defect is responsible, at least in part, for diminished serum concentrations of GH and IGF-1 in that condition [9]. Calorie/protein deprivation in humans results in large increments in the 24-hour integrated serum GH concentration and GH pulsatile secretion frequency caused by augmented GRF release despite a reduction in serum IGF-1 concentration [10, 11]. Resistence of IGF-1 production to hepatic GH action is present in both prolonged fasting and protein deprivation, with the defect in fasting attributable to a reduction in GH-receptor number and to a post-receptor defect in protein restriction [12, 13]. Since prolonged fasting is accompanied by chronic metabolic acidosis and potassium depletion, and since chronic metabolic acidosis is commonly accompanied by potassium depletion (such as renal tubular acidosis types 1 and 2, diarrhea, NH_4Cl ingestion), investigation of the effect of chronic metabolic acidosis on the GH/IGF-1 axis in humans may be of substantial pathophysiological and clinical importance.

Accordingly, the present studies were designed to evaluate the GH/IGF-1 axis prior to and following the experimental induction of chronic mineral acidosis in normal human subjects under conditions of constant dietary intake.

Methods

The protocol was designed to measure the effects of chronic NH_4Cl-induced metabolic acidosis on the growth hormone-IGF-1 endocrine axis in normal humans. Six normal male subjects were examined during metabolic balance studies. None were smokers nor were taking any drugs before and during the study. They

Received for publication May 28, 1996
and in revised form August 21, 1996
Accepted for publication August 22, 1996

Table 1. Effect of chronic NH_4Cl administration on fasting blood acid-base and plasma electrolyte composition

	H^+	$PaCO_2$	HCO_3^-	Unmeasured anions	Na^+	K^+	Cl^-	Creatinine clearance
	nmol/liter	*mm Hg*	*mmol/liter*					*ml/sec*
Control	38.2 ± 0.7	41.0 ± 0.9	25.0 ± 0.4	18.0 ± 0.5	142 ± 0.5	4.2 ± 0.1	103 ± 1	2.05 ± 0.08
Acidosis	48.0[a] ± 0.9	31.2[a] ± 1.4	15.5[a] ± 0.9	17.5 ± 0.8	141 ± 0.9	3.7[a] ± 0.1	113[a] ± 2	2.00 ± 0.06

[a] $P < 0.005$

ingested a constant metabolic diet for > five days before the study (prefeeding phase) and during the two study periods (control and acidosis).

To characterize the acid-base and electrolyte status, 24 hour urines were collected and fasting arterialized blood samples [14] were obtained in a heparin-coated syringe from a heated hand or forearm vein at 7 a.m. Blood samples were accepted if partial pressure of oxygen was > 70 mm Hg (9.3 kPa). For hormonal analysis, daily blood samples were obtained at 7 a.m. and samples were kept on ice until centrifuged at 4°C. The serum samples were stored at −30°C until analyzed. During this study the TSH response to the intranasal administration of 2 mg TRH was determined and reported elsewhere [15].

All subjects volunteered for the study, were paid for their participation from institutional resources, and gave informed consent. The study protocol was approved by the ethics committee of the Kantonsspital St. Gallen, Switzerland.

Experimental design

After establishment of the metabolic steady state [daily variation of plasma bicarbonate concentration by no more than 1.5 mmol/liter and by < 3 mm Hg (0.4 kPa) for $PaCO_2$], the growth hormone response to synthetic growth hormone releasing factor (GRF 1-44; Sanofi Winthrop, 1 μg/kg body wt as an i.v. bolus) was determined. Forty-five minutes after administration of GRF, octreotide (Sandoz, Basle, Switzerland) was given (50 μg as an i.v. bolus, followed by a constant infusion of 50 μg/hr). During this study the TSH response to the intranasal administration of 2 mg of TRH was also determined [15]. The TRH test was performed at least 48 hours before the GRF test. Two days after the GRF test, the response of serum IGF-1 concentration to the subcutaneous administration of human recombinant growth hormone (0.1 U/kg body wt every 12 hr for 48 hr; Genotropin, Kabi Pharmacia, Sweden) was determined.

The control period lasted until IGF-1 serum concentrations for at least two consecutive days were not different from the IGF-1 concentrations both before the GRF test and before growth hormone administration, respectively. IGF-1 serum concentration fell to basal levels in all subjects by 72 hours after the last dose of growth hormone. After the control period, metabolic acidosis was induced by oral administration of NH_4Cl (4.2 mmol/kg body wt/day) in gelatin capsules in six divided doses. The GRF test was repeated on day 5 of the acidosis period. The TRH test was repeated on day 6 of the acidosis period [15]. At the end of day seven of acidosis, growth hormone administration was repeated (0.1 U/kg body wt every 12 hr for 48 hr) and the IGF-1 response determined. Observations of IGF-1 concentration were continued for 72 hours after the last dose of growth hormone administration.

Analytical procedures

Analysis of plasma and urine electrolyte and acid-base composition was performed as described previously [16]. IGF-1 was determined radioimmunometrically after acid-ethanol extraction of serum [17]. IGF-BP3 was determined radioimmunometrically in serum using an antibody against pure acid-stabile 53 kDa binding subunit extracted from serum Cohn fraction IV [18]. Growth hormone in serum was measured using an IRMA [19].

Calculations

Serum half-life (t 1/2) was calculated from the decay rate of serum growth hormone concentration using a monoexponential regression model [20]. In the control period, the t 1/2 was calculated both for the decrease after achievement of peak GH concentration (at least 3 consecutive GH concentrations were decreasing) and for the GH concentrations following intravenous administration of octreotide (Sandoz; 45 min post-GRF, 50 μg as an i.v. bolus followed by an infusion of 50 μg/hr). In the acidosis period, t 1/2 was calculated for the decrease following the time points when GH concentration had reached the same level as in the control period (peak concentration and after octreotide).

The metabolic clearance rate (MCR) for GH (ml/kg/min) was estimated using the monoexponential growth hormone t 1/2 and assuming an average volume of distribution (DS) of 8.4%, according to the following equations [21]:

$$MCR = (DS)(\ln 2/t\ 1/2)$$

$$DS = 0.084 \times wt\ (kg)$$

Results are reported as means ± SEM unless stated otherwise. Statistical significance was determined by Student's two-tailed *t*-test for paired data.

Table 2. Effect of chronic NH_4Cl administration on urinary acid-base and electrolyte excretion

	pH	Net acid	Na^+	K^+	Cl^-
	U	*mmol/24 hr*		*mmol/24 hr*	
Control	6.10 ± 0.08	47.9 ± 4.2	134 ± 7	84 ± 5	141 ± 6
Acidosis	5.23[a] ± 0.14	318.4[a] ± 19.2	138 ± 8	87 ± 7	457[a] ± 19

[a] $P < 0.005$

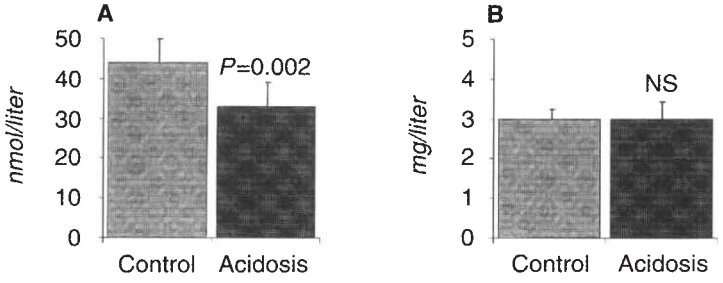

Fig. 1. *Effect of chronic metabolic acidosis on serum IGF-1 (A) and IGF-BP3 (B) concentrations in humans.* Metabolic acidosis was induced by administration of NH_4Cl (4.2 mmol/kg body wt/day).

Results

NH_4Cl administration resulted in sustained hyperchloremic metabolic acidosis (Table 1). Plasma bicarbonate concentration decreased significantly from 25.0 ± 0.4 in the control period to 15.5 ± 0.9 mmol/liter during acidosis ($P < 0.001$). Creatinine clearance was not affected significantly by acidosis (2.05 ± 0.08 vs. 2.00 ± 0.06 ml/second, NS). NH_4Cl administration induced a significant fall in plasma potassium concentration from 4.2 ± 0.1 to 3.7 ± 0.1 mmol/liter ($P < 0.0025$), consistent with our previous observation of renal hypokalemia and negative sodium balance in chronic metabolic acidosis [16]. The effects of chronic NH_4Cl administration on urine acid-base and electrolyte compostion are shown in Table 2. All subjects tolerated NH_4Cl well and lost weight (2.3 ± 0.8 kg for the 7-day acidosis period before growth hormone administration, $P < 0.025$) as reported previously [16].

As illustrated in Figure 1, serum IGF-1 concentration fell significantly from 44.5 ± 6.3 nmol/liter during control period to 33.4 ± 6.0 nmol/liter ($P = 0.002$) after seven days of metabolic acidosis. Figure 1 also illustrates that the serum concentration of IGF binding protein 3 was not affected significantly by acidosis: 3.0 ± 0.2 mg/liter during control and 3.0 ± 0.3 mg/liter during acidosis. The steady-state basal serum GH concentrations increased slightly, but insignificantly, from 0.15 ± 0.05 to 0.35 ± 0.05 μg/liter at 0700 (a.m.) and from 0.10 ± 0.02 to 0.40 ± 0.05 μg/liter at 1700 (p.m.) during chronic acidosis.

To further evaluate the level of inhibition of metabolic acidosis on the GH/IGF-1 endocrine axis, the GH response to GRF administration (1 μg/kg body wt as an i.v. bolus) was examined. As illustrated in Figure 2, the GRF-stimulated GH response was significantly greater during chronic metabolic acidosis as compared to control. This exaggerated response is evidence for a loss of normal feedback inhibition owing to reduced IGF-1 levels. The results of this experiment are most easily explained by a primary suppression of hepatic IGF-1 synthesis/secretion.

Based on the results of the GRF test and basal GH serum concentrations, the integrated 24-hour secretion rate of GH would be expected to be increased in response to acidosis. IGF-1 suppression might, therefore, be the result of hepatic GH insensitivity. To examine this possibility, we evaluated the IGF-1 response to administration of high doses of GH (0.1 U/kg body wt every 12 hr for 48 hr) during control and acidosis periods. Figure 3 illustrates that the IGF-1 response to GH administration was significantly blunted during the acidosis period. This effect could be due to a GH receptor-mediated resistance to the hepatic action of GH and/or an independent post-receptor suppressive effect of metabolic acidosis on the hepatic synthesis of IGF-1. A potential role for potassium depletion (Tables 1 and 2) in mediating the present finding of a blunted IGF-1 response to GH administration

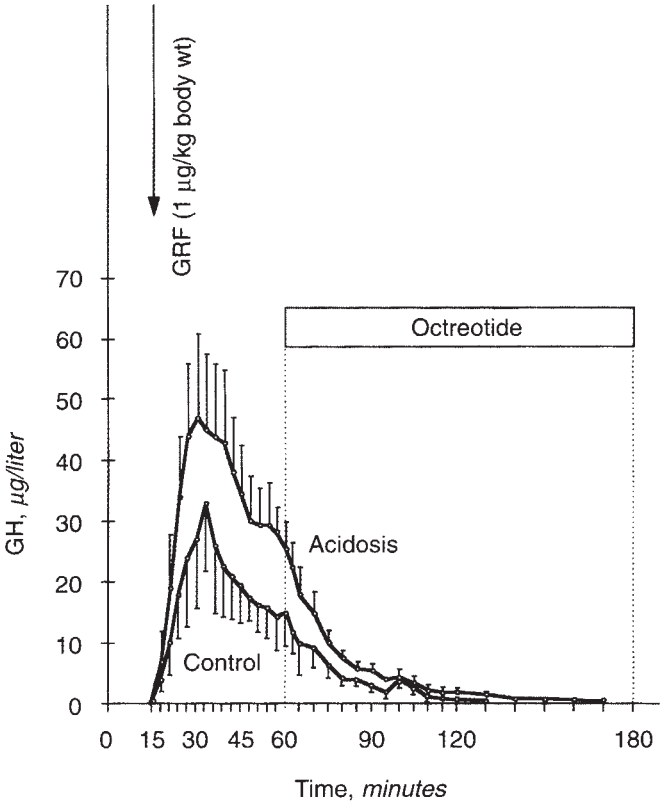

Fig. 2. *Growth hormone response to growth hormone releasing factor (GRF, 1 μg/kg body wt, i.v. bolus).* Forty-five minutes after GRF, octreotide was administered as indicated (time point: 60 min).

cannot be assessed at present since there are no reports of this response under conditions of potassium depletion in any species.

The GRF test was also used to estimate the endogenous half-lives and metabolic clearance rates (MCR) of GH before and during secretory inhibition by octreotide. Since MCR decreases and serum half-life increases with increasing serum concentrations of GH [20], half-life and MCR during acidosis were estimated from those portions of the decay curves corresponding to identical serum GH concentrations. The results of these determinations, shown in Table 3, demonstrate that there were no significant differences in the serum half-life and MCR between control and acidosis periods. These results provide evidence that the decreased serum IGF-1 levels in chronic metabolic acidosis cannot be explained by decreased half-life or increased MCR of serum GH.

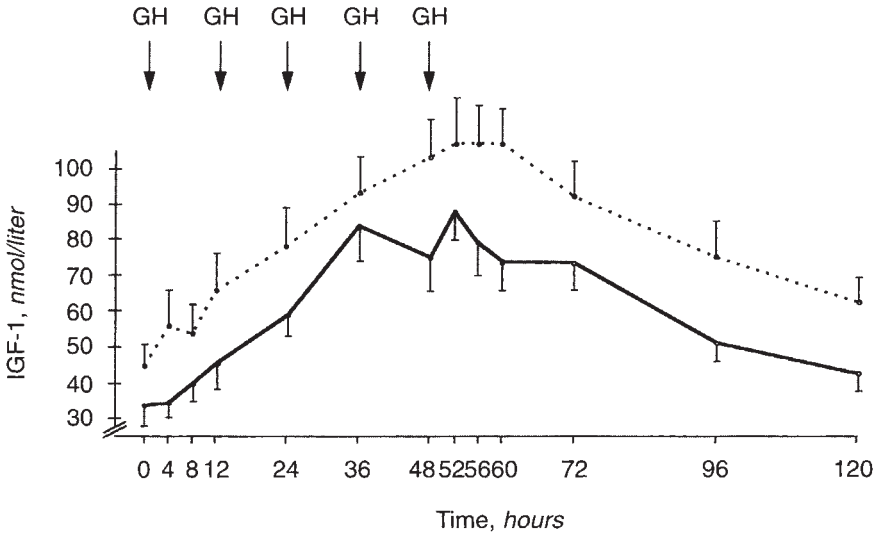

Fig. 3. *IGF-1 response to growth hormone administration in metabolic acidosis.* Symbols are: (. . . .) control; (———) acidosis. Growth hormone was administered as indicated (0.1 U/kg body wt/dose). $^{*}P < 0.05$

Table 3. Effect of metabolic acidosis on serum half-time (t 1/2) and metabolic clearance rate (MCR) of GH

	Cp	Coct	t 1/2p	t 1/2oct	MCRp	MCRoct
	μg/liter		*min*		*ml/min/m²*	
Control	36 ± 10	15 ± 5	21 ± 5	10 ± 3	77 ± 9	161 ± 34
Acidosis	45 ± 12[a]	28 ± 7[a]	20 ± 4	11 ± 2	74 ± 8	154 ± 22

Serum half-time (t 1/2) and MCR were calculated for identical serum concentrations during both study periods (that is, two GH serum concentrations measured during control period were chosen for comparison: GH peak concentration after GRF administration and GH concentration at the beginning of octreotide administration). Abbreviations are: Cp, the peak GH concentration after GRF administration; Coct, GH concentration at the beginning of octreotide administration; MCRp,oct, metabolic clearance rate after peak concentration and after concentration at the beginning of octreotide administration, respectively.

[a] $P < 0.05$

Discussion

The present studies demonstrate that induction of chronic metabolic acidosis in human subjects results in a significant reduction of serum IGF-1 concentration in association with an apparent insensitivity to the hepatocellular action of GH, as evidenced by the blunted IGF-1 response to administration of pharmacological doses of GH. The acidosis-induced reduction of serum IGF-1 concentration was associated with an accentuated increase in serum GH concentration in response to GRF administration, as evidence for a sustained loss of normal feedback inhibition of pituitary GH secretion owing to reduced circulating levels of IGF-1.

A central novel finding of the present studies is that CMA induces apparent insensitivity to GH in humans (Fig. 3). These studies, however, were not designed to analyze the mechanism(s) of this insensitivity and studies at the cellular level are needed to determine whether alterations in GH receptor regulation, postreceptor signal transduction defects and/or defects in IGF-1 synthesis [22] are primarily responsible.

The pathophysiology of acidosis-induced decrements in serum IGF-1 concentration appears similar to that reported during prolonged fasting in normal subjects, where increased 24-hour integrated serum GH concentration and GH pulsatile secretory frequency have been reported [10, 11]. However, the GH secretory response to GRF has not been reported during fasting. Resistence of IGF-1 production to hepatic GH action occurs in both prolonged fasting and in protein deprivation, with the defect in fasting attributable to a reduction in GH receptor number [12] and to a post-receptor defect in protein restriction [13]. Reductions in hepatic GH receptor mRNA and hepatic IGF-1 mRNA expression have been reported for NH_4Cl-induced metabolic acidosis in rats [6], although the reduction was not observed when compared to pair-fed non-acidotic rats. The reduction in hepatic IGF-1 mRNA has not been confirmed [23].

The frequency and magnitude of GH secretion in chronic metabolic acidosis in adult humans has not been reported, but is expected to be increased based on the present finding of a greatly augmented GRF-induced GH response with its implications for diminution of pituitary-level feedback inhibition. A brief period (3 hr) of NH_4Cl loading has been reported to result in significant elevation in plasma GH concentration in normal subjects [24]. The present finding of an elevated mean value, tendentiously increased but statistically unchanged basal 7 a.m. and 5 p.m. serum GH concentrations in acidotic subjects, is not inconsistent with an augmented 24-hour integrated GH secretion rate inasmuch as GH secretion occurs in brief, episodic pulses throughout the day.

The mechanism whereby decrements in serum IGF-1 concentration in diverse conditions result in enhanced GH secretion has been investigated both *in vitro* and *in vivo* with evidence provided for IGF-1-induced negative feedback inhibition of GH secretion at both pituitary and hypothalamus [10, 25, 26]. Importantly, it has been demonstrated that fasting-induced augmented GH secretion in normal subjects occurs under circumstances of diminished negative feedback regulation owing to reduced IGF-1 levels [10].

Since chronic mineral acidosis and other causes of chronic metabolic acidosis result in potassium depletion, the possibility might be considered that the acidosis-induced abnormalities in the GH/IGF-1 axis are secondary to disordered potassium metabolism. This possibility is unlikely based on the observation that the

serum IGF-1 levels are reduced significantly in potassium depleted rats in association with a diminished rather than acidosis-induced augmented GH response to GRF administration [9].

In contrast to the findings in protein deprivation [27], IGFBP-3 concentrations were unchanged during metabolic acidosis when total serum IGF-1 concentrations were reduced. Thus, in metabolic acidosis free IGF-1 levels are likely to be reduced.

Chronic metabolic acidosis results in primary hypothyroidism in humans (unpublished observations) [15]. Since hypothyroidism results in decreased GH secretion in rats and in decreased plasma IGF-1 concentrations in humans [28] and rats [29], it might be predicted that the acidosis-induced alterations in the GH/IGF-1 axis are mediated, at least in part, by concurrent hypothyroidism. This is unlikely for several reasons: In both hypothyroid rats [30, 31] and humans [32], the serum GH response to GRF administration is blunted, not enhanced as shown in the present study. Moreover, the serum IGF-1 response to GH administration in human hypothyroidism is reported to be unimpaired [33] rather than the blunted IGF-1 response demonstrated in the acidotic subjects of the present studies. Human hypothyroidism has also been reported to result in impaired bioactivity of circulating IGF-1 as assessed with an IGF-1 cartilage sulfation bioassay [28], but it remains to be investigated whether acidosis results in impaired IGF-1 bioactivity.

In summary and conclusion, the central findings of the present studies show that metabolic acidosis in humans results in a significant decrease in serum IGF-1 concentration that is independent of a change in IGFBP-3 concentration and related to a demonstrable insensitivity to the hepatocellular action of GH. The primary hepatocellular defect within the GH/IGF-1 axis results in diminished negative feedback inhibition of IGF-1 on GH as evidenced by the enhanced serum GH response to GRF administration. The present finding of an unchanged MCR for GH in chronic metabolic acidosis will enhance interpretation of future studies in which pulsatile serum GH concentration profile is determined in this disorder. The unchanged metabolic clearance rate will permit determination of alterations in GH secretion rate from serum GH concentrations in acidotic subjects.

Acknowledgments

The authors wish to thank J. Girard (Basle, Switzerland) for performing IGF-1, GH and IGF-BP 3 at a reduced price. These studies were supported by institutional funding from the Klinik B für Innere Medizin. The authors also acknowledge the able assistance of M. Koeppel.

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